INTRODUCTION

Within a few days of birth, striking changes occur in the structure of the adrenal gland. At full term, the suprarenal gland consists of both definite and foetal cortex with a central zone of medulla surrounding large blood sinuses. The outermost layer of the foetal gland remains and differentiating into the characteristic basophilic post-natal cortex. During the first year of life, the whole gland shrinks due to the rapid disappearance of foetal cortex. The foetal cortex rapidly undergoes involution, often with haemorrhage and necrosis, and with the appearance of abundant fat-staining material. The involution starts in the foetal zone by the end of 2nd week after birth with the complete establishment by the end of 1st month and lasting throughout 1st year of life. This is succeeded by replacing the connective tissue surrounding the medulla and cortex as a narrow strip of fibrous tissue. The gland acquires adult appearance by 8th year. Cells in the definitive cortex are smaller than those in foetal cortex. The cells of foetal cortex are larger with abundant

Background: Adrenal gland is a lifesaving endocrine gland of the human body. Knowledge of normal microscopic anatomy of the Adrenal gland facilitates Pathologists to correlate and make the differential diagnosis of various adrenal glands associated clinical conditions on histopathological examination. Aims and Objective: To study histological features of human adrenal gland in the post-natal period at different ages. Materials and Methods: Present study is conducted with 98 human adrenal glands from 49 unclaimed dead bodies during an autopsy and 22 human adrenal glands from 11 cadavers during routine dissection in S.V Medical College, Tirupati, which were utilized after obtaining consent from the close kith and kin. During the autopsy/dissection, the samples collected were from 2 Years to 64 years of age individuals of both sex with typical external features and divided into three groups. The first group consisted of less than 25 years of age. The second group consists of ages between 26 years to 50 years. The third group included 51 years and above, and all the specimens are processed for histological staining and observed under the microscope. Results: At 2 years well-developed capsule with large vessels and well-differentiated zones of definitive cortex and medulla were identified. The thick muscular coat of the central vein was identified at 20 years. At 36 years & 45 years, cellular architecture in various zones is very well differentiated. At 65 years, the cortico-medullary demarcation was irregular, and vascularity decreased. Conclusion: Histological features of pre-pubertal, adult, and old age group sections helps in the understanding of post-natal changes in the growth of the Suprarenal gland and its functions.

Key words: Post-natal; Suprarenal gland; Cortex; Medulla; Reticulin stain
eosinophilic cytoplasm and vesicular nuclei. During the involution process, the foetal cortex cells are swollen and are replaced by a temporary fibrous tissue that will be seen adjacent to zona fasciculata and persists up to 3rd year. The zona fasciculata is differentiated by 3rd month, and by 4th month, the reticularis is differentiated, and the medullary cells could be identified. The outer zone of polygonal true or definite cortex cells invade the inner degenerating foetal cortex and replace it.\(^2\)\(^a\)

According to Ernest et.al. (1996)\(^4\) the definitive cortex of the new-born is a thin subcapsular band of 0.1 to 0.2 mm thick. The zonation of the permanent cortex is not evident until the end of the first month of life. Some evidence of zona glomerulosa and zona fasciculata of the cortex and pale staining chromaffin cells with vesicular round to oval nuclei of medulla will be apparent at this time. After birth, the foetal cortex regresses rapidly except for its outermost part, differentiated into zona reticularis. The adult structure of the cortex is not achieved until puberty.\(^5\)

Bocian-Sobkowska (2000)\(^6\) reported a post-natal decrease in adrenal volume. According to them, the post-natal decrease in adrenal volume was mainly due to rapid fall of foetal zone (FZ) volume (from 70 to 3\% of total adrenal volume), which can be divided into two phases: rapid phase (from birth to the end of the second week) and a slow phase from the 3rd week onwards. In their study, Involution was accompanied by an increase of zona glomerulosa (ZG) (from 10 to 25\% of total adrenal volume), zona fasciculata (ZF) (from 10 to 38\%) and zona reticularis (ZR), volume (from 1 to 23\%). During the whole investigated period the volume of the medulla remained constant. The volume fraction of stroma (connective tissue and blood vessels) was highest at the beginning of the first post-natal week and then decreased rapidly at the end of the 2nd week, with the most pronounced changes in the foetal zone and medulla.

The general histological appearance of the adrenal gland is typical of an endocrine gland, in which cells of both cortex and medulla are grouped in cords along capillaries. A dense connective tissue capsule that covers the adrenal gland sends thin septa to the gland’s interior as trabeculae. The stroma consists mainly of a rich network of reticular fibres that supports the secretory cells.

More distinct zonation is apparent in the adult cortex. According to Lack and Kozakewich (1990).\(^7\) Zona-gglomerulosa (ZG) contains small ball-like aggregates immediately beneath the capsule and the lipid rich cells and have pale-staining cytoplasm. According to Ernest et.al. (1996)\(^4\) ZG is poorly defined or discontinuous and is never prominent in the normal adrenal gland. Zona fasciculata (ZF) comprises 70\% of the normal adult adrenal cortex and consists of radially arranged cords of lipid rich cells that merge with zona reticularis (ZR). It consists of compact or lipid depleted cells with eosinophilic cytoplasm and arranged in small anastomosing cords.\(^4\)

The junction between cortex and medulla is generally smooth. Still, cortico-medullary intermingling with tongue-like extensions of cortical cells into medulla or discontinuous islets of cortical cells among chromaffin cells are considered normal.\(^4\)

According to standard text-books of histology, the adrenal cortex is subdivided into three concentric layers whose limits are usually not sharply defined in humans. Outermost Zona Glomerulosa (ZG) -15 \%. (outer 1/5\text{th} of cortex). Middle Zona Fasciculata (ZF)-65-80 \%. (middle 3/5\text{th} of cortex). Inner Zona Reticularis (ZR)-10 \%. (inner 1/5\text{th} of cortex).\(^8\)

**Zona Glomerulosa (ZG)** is directly under the connective capsule. Cells are arranged into inverted “U” shaped formations/acinus like groups/ ovoid groups/ arched columns/ irregular clusters separated by delicate fibrous trabeculae continuous with the fibro collagenous capsule. Both the trabeculae and inner capsule contain prominent capillaries. Cells are polyhedral/pyramidal/columnar with basophilic cytoplasm and deeply stained round nuclei.\(^9\)-\(^13\)

**Zona fasciculata (ZF)** in the middle and broadest of the three cortical zones. Cells are arranged in vertical columns/ radial plates with one / two cell thickness separated by fine strands of collagen and wide bore capillaries. Cells are polyhedral in shape with abundant and pale staining cytoplasm due to large number of lipids, mitochondria, and smooth endoplasmic reticulum with the vesicular nucleus. They are also called ‘spongiocytes’ due to vacuolization and are rich in vitamin C. lipids and cholesterol.\(^9\)-\(^13\)

**Zona reticularis (ZR)** is a thin innermost zone of the adrenal cortex, lies between Zona fasciculata and medulla. Cells are arranged in an irregular network of branching cords and clusters, separated by numerous wide diameter capillaries. Cells are small with less dark staining cytoplasm with fewer lipid droplets. Brown lipofuscin pigment is also present as granules.\(^3\)-\(^13\)

**Adrenal medulla** is not sharply demarcated from the cortex. Cells are arranged in cords /clumps and supported by a reticular fibre network. It consists of chromaffin cells, ganglion cells, capillaries & sinusoids. The chromaffin cell is tall columnar with basophilic cytoplasm containing membrane-bound granules. These granules stain brown when treated with potassium dichromate, known as a chromaffin reaction. Cells containing noradrenalin exhibit
a strongly positive chromaffin response than adrenalin secreting cells. Ganglion cells are sympathetic neurons seen singly or in small groups. They exhibit vesicular nucleus, prominent nucleolus and a small amount of peripheral chromatin.9-13

Cortical nodularity presenting fibrous tissue covered capsule that is continuous with adjacent capsule and enclosing cortical cells that become continuous with cortex was observed with increasing frequency in aged glands and hypertension; and diabetes mellitus.4,14,15

MATERIALS AND METHODS

A total of 120 suprarenal glands obtained from 60 cadavers were included in this study. A total of ninety-eight human adrenal glands from forty-nine unclaimed dead bodies which were under post-mortem examination in department of Forensic medicine. S.V. Medical College, Tirupati and 22 human adrenal glands from 11 cadavers during routine dissection for academic purposes, in the Department of Anatomy, S.V Medical College were utilized after obtaining consent from the close kith and kin. During post-mortem examination / dissection, the adrenal glands were collected after recording its location, relation with the kidney.

Collected adrenal glands were preserved in 10% Formalin and then sections were taken from representative samples and subsequently subjected to tissue processing and section cutting. Sections were stained with Haematoxylin and Eosin, Giemsa, Reticulin stains and the histological features were analysed. The samples collected were from 2 Years to 64 years age individuals of both sex with typical external features and divided into three groups. The first group consisted of less than 25 years of age. The second group between 26 years to 50 years. The third group included 51 years and above.

RESULTS

Observations in less than 25 years age 1st group
In this age group histological sections of 2 years, 14 years, and 20 years. Individuals’ adrenal glands were observed in Figures 1 and 2). At two years, the folded appearance of cortex with a thick capsule could be identified (Figure 1). At 20 years, there is an increase in the thickness of the vessel wall (Figure 2). Due to extensive folding of cortex medulla is seen as a scattered mass extending into a part of the cortical fold (Figures 1 and 3). Lightly stained cortex and darkly stained medulla could be identified even at lower magnification (Figure 3), and there is an increase in the thickness of medulla. At a higher magnification in the less than 25 years group, clearly differentiated zones of adult or definitive cortex and medulla could be identified (Figure 4-7). The zona glomerulosa presented cells that are arranged in ball-like/ glomerulus aggregates. These...
cells are darkly stained and basophilic in appearance and presented a profoundly stained nucleus (Figure 4 and 6). In zona fasciculata, the cells are arranged in the form of radial cords with sinusoidal capillaries in between. These cells are lightly stained, and the nuclei are vesicular in appearance, and the cytoplasm is basophilic and appeared like lipid-laden cells (Figure 4, 6, 7). The zona reticularis is seen as a thin layer and presented condensed basophilic cells in the form of a small band (Figure 4, 6, 7). The cortico-medullary transition was smooth in this age group (Figure 5 -7). Medullary cells are large polyhedral with the vesicular nucleus and fine granules with Giemsa staining (Figure 5). The thick muscular coat of central vein could be identified at 20 years. (Figure 2).

**Observations in 26 – 50 years age 2nd group**

In this age group, histological sections of 28 years, 36 years, 45 years and 50 years. Individuals’ adrenal glands were observed in Figure 8 and 9). In this age group the capsule is thick (Figure 8), and it presented clear zonation of cortex and medulla with central vein (Figure 9). The three zones of the cortex the ZG, ZF and ZR are well differentiated of which fasciculate is the widest, followed by glomerulosa and reticularis (Figure 10-13). The cellular architecture in various zones is very well differentiated in this group than...
in the less than 25 years age group. The medulla presented a large number of sinusoids (Figure 12). The wall of the central vein is more thickened than in previous groups (Figure 13).

**Observations in > 50 years age 3rd group**

In this group, a section of 62 years and 65 years were observed. The cells of the cortex and medulla could be identified (Figure 14-16). Though various zones of the cortex could be identified, the cells of ZG presented scattered distribution (Figure 14) of cells with rounded nuclei and less cytoplasm. Cells of ZF are larger than those of zona glomerulosa and are lightly stained due to lipid deposition (Figure 15). Cells of ZR presented signs of pyknosis and cell death (Figure 16). The corticomedullary demarcation was irregular (Figure 16). Medulla presented cells filled with dark granules. In this age group, the vascularity is less when compared to previous groups, as is evident from the size and number of capillaries.

Reticulin stain at 2 years and 65 years. Gland section demonstrated regular, the parallel arrangement of reticular and collagen fibres in relation to the capsule and longitudinal arrangement corresponding to cuboidal cortical cells that are arranged in columns between blood-filled sinusoids.
(Figures 17-18). The medullary reticular fibres are arranged in an irregular fashion when compared to cortical reticulin. With the increase in age, there is an increase in the thickness of reticular fibres.

DISCUSSION

Post-natal developmental histology in less than 25 years age 1st group
At 2 years well, developed capsule with large vessels that are entering into the substance of the gland, clearly differentiated zones of adult or definitive cortex and medulla was identified. A well-defined medulla could be identified, as was stated by Crowder (1957). Well-differentiated zona glomerulosa and fasciculata and a thin rim of zona reticularis were observed at 2 years. The cells of ZG are darkly stained and basophilic in appearance with the deeply stained nucleus. The cells of ZF are lightly stained, and the nuclei are vesicular in appearance. The cytoplasm of cells of ZF is basophilic and appeared like lipid-laden cells. ZR presented condensed basophilic cells. Cortico-medullary transition was smooth in less than 25 years age group. Medullary cells are large polyhedral with a vesicular nucleus and fine granules. The thick muscular coat of central vein could be identified at 20 years. The...
stroma presented a fine network of reticular fibres that are seen with reticulin stains. Of the three zones of cortex, ZF is the widest. According to standard text-books of histology, the adrenal cortex is the limits of three zones of cortex are usually not sharply defined in humans. But in the present study, they were well defined in the less than 25 years group.\textsuperscript{2,13}

The observations on histological features are in agreement with those reported in the literature,\textsuperscript{1-4} and the gland acquired adult characters by 20\textsuperscript{th} year in the present study, which is in variance to that reported in the literature where these adult features were observed at 8 years.\textsuperscript{1,3}

\textbf{In 25 – 50 years age 2\textsuperscript{nd} group}

In general, the observations are in agreement with those reported in the literature or text-books.\textsuperscript{3-13} In this age group, the capsule is thick, and it presented clear zonation of cortex and medulla with central vein. The three zones of the cortex the ZG, ZF and ZR are well-differentiated, of which fasciculata is the widest (3/5\textsuperscript{th}) followed by ZG (1/5\textsuperscript{th}) and ZR (1/5\textsuperscript{th}).

The cellular architecture in various zones is very well differentiated in this group than in the less than 25 years age group. Cortico-medullary demarcation was smooth. The medulla presented a large number of sinusoids. The wall of the central vein is more thickened than in the prenatal group. The observations in this group are in agreement that stated by Lack and Kozakewich (1990)\textsuperscript{7} Siddiqua D et al. (2014).\textsuperscript{16} According to Ernest et al, (1996)\textsuperscript{4} zona glomerulosa is poorly defined or discontinuous and is never prominent in the normal adrenal gland. Still, the observations in the present study are not in agreement.

\textbf{In > 50 years age 3\textsuperscript{rd} group}

Though various zones of the cortex could be identified, the cells of ZG presented the scattered distribution of cells with rounded nuclei and less cytoplasm. Cells of ZF are larger than those of ZG and are lightly stained due to lipid deposition. Cells of ZR presented signs of pyknosis and cell death. The cortico-medullary demarcation was irregular and is in agreement with the observation reported by Ernest et al (1996).\textsuperscript{4} Medulla presented cells filled with dark granules, which is also mentioned in literature.\textsuperscript{4} In this age group, the vascularity is less when compared to previous groups, as is evident from the size and number of capillaries.

Reticulin stain at 2 years and 65 years. Gland section demonstrated regular, the parallel arrangement of reticular fibres in relation to a capsule and longitudinal arrangement corresponding to cortical cells that are arranged in columns between blood-filled sinusoids. The medullary reticulin fibres are arranged in an irregular fashion when compared to cortical reticulin. With an increase in age, there is an increase in the thickness of reticular fibres.

\textbf{CONCLUSION}

The disappearance of fetal zone and differentiation of cortex and medulla with cortical zonation occurs at the age of 2 years. The gland acquired adult characters by 20\textsuperscript{th} year. A well-differentiated zonation and cellular architecture occurs at the age of 25 years. Signs of degeneration and reduced vascularity occur at 65 years of age. Reticular fibres orientation at the difference in capsule, cortex and medulla and increase in their thickness happens with advancing age. Observations on age-related changes in the microscopic structure of pre-pubertal, adult, and old age suprarenal gland sections helps in the understanding of progressive changes in the adrenal gland. Adrenal gland normal histological study is important to diagnose and differentiate various clinical conditions during the histopathological examination.

\textbf{REFERENCES}

Authors Contribution:

MR- Concept and design of the study; interpreted the results, prepared the first draft of the manuscript and critical revision of the manuscript; SD- Design of the study, Histological findings, and interpretation, preparation of the manuscript and revision of the manuscript; RK- Histological findings analysis and interpreted; reviewed the literature and manuscript preparation; SR- Concept, and coordination of the overall study.

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